S10.17 Evaluation of antioxidant properties of some pyrazolo[3,4-*d*]pyrimidines derivatives and their effects on mitochondria bioenergetics

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In recent years, pyrazolopyrimidines and related fused heterocycles have been identified as bioactive molecules. They are known to function as CNS (Central Nervous System) depressants, neuroleptic agents, and as tuberculostatic. Pyrazolo[3,4-*d*]pyrimidines were identified as a general class of adenosine receptors. The aim of this work was to determine the antioxidant properties of some novel pyrazolo[3,4-*d*]pyrimidine derivatives once oxidative stress is thought to play an important role in numerous degenerative or chronic diseases, such as atherosclerosis and cancer. Our results show that some of these compounds have good antioxidant when using ABTS and DPPH methods. Furthermore, membrane protection against *tert*-butylhydroperoxide oxidation was observed by the decrease in the TBARS produced when mitochondrial membranes were pretreated with those compounds. We also evaluate their effect on mitochondrial bioenergetics. State 4, state 3 respiration, and mitochondrial membrane potential will be studied in the presence of the more antioxidant efficient compounds.

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S10.18 Interaction of mitochondria-targeted antioxidants with cells in culture

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Ubiquinone or plasoquinone conjugated via decane linker with triphenyl phosphonium cation (MitoQ and SkQ1, respectively) are shown to be powerful antioxidants in isolated mitochondria. We found that plastoquinone conjugated with positively charged fluorophore rhodamine-19 (SkQR1) selectively accumulates in mitochondria of HeLa cells and human fibroblasts, reaching plateau at 2 h. Uncoupler FCCP suppresses the accumulation and stimulates the release of SkQR1. Incubation with nanomolar SkQ1 or SkQR1 for 2 h prevents oxidation of glutathione and fragmentation of mitochondria induced by H_2O_2. However, 2 h incubation does not result in resistance of HeLa cells and human fibroblasts to H_2O_2 or other prooxidants (menadione, paraquat). The protective effect becomes pronounced only after 24 h incubation and disappeares during 48 h after removal of quinones in parallel with SkQR1 release from the cells. Prolonged (6–7 d) incubation with 1–20 nM SkQ1 causes strong resistance against oxidative stress, MitoQ being 100 times less effective. Simultaneously, the antioxidants promote structural and functional fusion of mitochondria increasing the size of electrically-united mitochondrial network. Staining of mitochondria with SkQR1 for 2 h revealed their significant heterogeneity while after prolonged incubation mitochondria become uniformly stained. It is suggested that slow development of the antiapoptotic effect is related to slow redistribution of mitochondria-targeted antioxidants between mitochondria in the cell.

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S10.19 Pro-oxidant and anti-oxidant properties of mitochondrial matrix-targeted ubiquinone MITOQ$_{10}$

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Addition of mitochondria-targeted coenzyme Q, MitoQ$_{10}$, to HEP-G2 cells with rotenone-inhibited mitochondrial Complex I sharply decreases rotenone-induced superoxide release to the matrix (J_m) and increases cell respiration. Thenoyltrifluoroacetone, a Complex II inhibitor, together with rotenone completely inhibited HEPG2 cell respiration and kept high J_m, while subsequent MitoQ$_{10}$ addition restored again respiration in an antimycin- (stigmatellin- and myxothiazol-)-dependent manner, but did not prevent high J_m. We conclude that MitoQ$_{10}$ is able to accept electrons prior to the rotenone-bound Q-site and that a reverse mode of Complex II is likely required to regenerate the reduced MitoQ$_{10}$H$_2$ back to MitoQ$_{10}$. Complex III inhibitors (antimycin, stigmatellin and myxothiazol) prevented high J_m in HEPG2 cells induced with either rotenone and MitoQ$_{10}$, or with rotenone, MitoQ$_{10}$, and thenoyltrifluoroacetone. MitoQ$_{10}$ alone increased basal J_m in HEPG2 cells highlighting also its *pro*-oxidant property. Described effects of all reagents combinations were more pronounced in J_m HEPG2 cells compared to H_2O_2 formation in isolated liver mitochondria. In conclusion, MitoQ$_{10}$ possesses a *pro*-oxidant role when added to intact mitochondrial respiratory chain, whereas its antioxidant role is striking when Complex I-derived superoxide generation increases due to retardation of electron flow within the Complex I.

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S10.20 Mitochondrial phospholipase iPLA2-dependent regulation of uncoupling protein UCP2

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We tested a hypothesis that reactive oxygen species (ROS)-dependent activation of mitochondrial phospholipases leads to an increase in respiration and to more intensive attenuation of mitochondrial ROS production due to UCP2-dependent uncoupling. This was tested using rat lung mitochondria and further verified using lung mitochondria isolated from UCP2-WT and UCP2-KO mice. Mitochondria with succinate as a substrate exhibited a steady increase in respiration which was further stimulated by low concentrations of *tert*-butyl hydroperoxide (TBHP, 5–25 μM). The observed respiration increase was fully inhibited by BSA, indicating the participation of free fatty acids, and by bromoenol lactone (BEL, 10 μM), a specific inhibitor of phospholipases iPLA2. The respiration was further partially inhibited by GTP, an inhibitor of UCP2. The TBHP-dependent increase in respiratory rate was not observed in lung mitochondria isolated from UCP2-KO mice. Parallel detection of H_2O_2 by Amplex Red revealed that neither BSA nor BEL caused a significant increase in mitochondrial H_2O_2 production under the given experimental